

[0263] Similarity analysis includes database search and alignment. Examples of public databases include the DNA Database of Japan (DDBJ); Genbank; and the European Molecular Biology Laboratory Nucleotide sequence Database (EMBL).

[0264] A number of different search algorithms have been developed, one example of which are the suite of programs referred to as BLAST programs. There are five implementations of BLAST, three designed for nucleotide sequences queries (BLASTN, BLASTX, and TBLASTX) and two designed for protein sequence queries (BLASTP and TBLASTN) (Coulson, *Trends in Biotechnology*, 12: 76-80 (1994); Birren, et al., *Genome Analysis*, 1: 543-559 (1997)).

[0265] BLASTN takes a nucleotide sequence (the query sequence) and its reverse complement and searches them against a nucleotide sequence database. BLASTN was designed for speed, not maximum sensitivity, and may not find distantly related coding sequences. BLASTX takes a nucleotide sequence, translates it in three forward reading frames and three reverse complement reading frames, and then compares the six translations against a protein sequence database. BLASTX is useful for sensitive analysis of preliminary (single-pass) sequence data and is tolerant of sequencing errors (Gish and States, *Nature Genetics*, 3: 266-272 (1993), herein incorporated by reference). BLASTN and BLASTX may be used in concert for analyzing EST data (Coulson, *Trends in Biotechnology*, 12: 76-80 (1994); Birren et al., *Genome Analysis*, 1: 543-559 (1997)).

[0266] Given a coding nucleotide sequence and the protein it encodes, it is often preferable to use the protein as the query sequence to search a database because of the greatly increased sensitivity to detect more subtle relationships. This is due to the larger alphabet of proteins (20 amino acids) compared with the alphabet of nucleotide sequences (4 bases), where it is far easier to obtain a match by chance. In addition, with nucleotide alignments, only a match (positive score) or a mismatch (negative score) is obtained, but with proteins, the presence of conservative amino acid substitutions can be taken into account. Here, a mismatch may yield a positive score if the non-identical residue has physical/chemical properties similar to the one it replaced. Various scoring matrices are used to supply the substitution scores of all possible amino acid pairs. A general purpose scoring system is the BLOSUM62 matrix (Henikoff and Henikoff, *Proteins*, 17: 49-61 (1993), herein incorporated by reference in its entirety), which is currently the default choice for BLAST programs. BLOSUM62 is tailored for alignments of moderately diverged sequences and thus may not yield the best results under all conditions. Altschul, *J. Mol. Biol.* 36: 290-300 (1993), herein incorporated by reference in its entirety, uses a combination of three matrices to cover all contingencies. This may improve sensitivity, but at the expense of slower searches. In practice, a single BLOSUM62 matrix is often used but others (PAM40 and PAM250) may be attempted when additional analysis is necessary. Low PAM matrices are directed at detecting very strong but localized sequence similarities, whereas high PAM matrices are directed at detecting long but weak alignments between very distantly related sequences.

[0267] Homologues in other organisms are available that can be used for comparative sequence analysis. Multiple alignments are performed to study similarities and differ-

ences in a group of related sequences. CLUSTAL W is a multiple sequence alignment package available that performs progressive multiple sequence alignments based on the method of Feng and Doolittle, *J. Mol. Evol.* 25: 351-360 (1987), the entirety of which is herein incorporated by reference. Each pair of sequences is aligned and the distance between each pair is calculated; from this distance matrix, a guide tree is calculated, and all of the sequences are progressively aligned based on this tree. A feature of the program is its sensitivity to the effect of gaps on the alignment; gap penalties are varied to encourage the insertion of gaps in probable loop regions instead of in the middle of structured regions. Users can specify gap penalties, choose between a number of scoring matrices, or supply their own scoring matrix for both the pairwise alignments and the multiple alignments. CLUSTAL W for UNIX and VMS systems is available at: <ftp://ebi.ac.uk>. Another program is MACAW (Schuler et al., *Proteins, Struct. Func. Genet.* 9:180-190 (1991), the entirety of which is herein incorporated by reference, for which both Macintosh and Microsoft Windows versions are available. MACAW uses a graphical interface, provides a choice of several alignment algorithms, and is available by anonymous ftp at: <ncbi.nlm.nih.gov> (directory/pub/macaw).

[0268] Sequence motifs are derived from multiple alignments and can be used to examine individual sequences or an entire database for subtle patterns. With motifs, it is sometimes possible to detect distant relationships that may not be demonstrable based on comparisons of primary sequences alone. Currently, the largest collection of sequence motifs in the world is PROSITE (Bairoch and Bucher, *Nucleic Acid Research*, 22: 3583-3589 (1994), the entirety of which is herein incorporated by reference.) PROSITE may be accessed via either the ExPASy server on the World Wide Web or anonymous ftp site. Many commercial sequence analysis packages also provide search programs that use PROSITE data.

[0269] A resource for searching protein motifs is the BLOCKS E-mail server developed by S. Henikoff, *Trends Biochem Sci.*, 18:267-268 (1993); Henikoff and Henikoff, *Nucleic Acid Research*, 19:6565-6572 (1991); Henikoff and Henikoff, *Proteins*, 17: 49-61 (1993); all of which are herein incorporated by reference in their entirety). BLOCKS searches a protein or nucleotide sequence against a database of protein motifs or "blocks." Blocks are defined as short, ungapped multiple alignments that represent highly conserved protein patterns. The blocks themselves are derived from entries in PROSITE as well as other sources. Either a protein or nucleotide query can be submitted to the BLOCKS server; if a nucleotide sequence is submitted, the sequence is translated in all six reading frames and motifs are sought in these conceptual translations. Once the search is completed, the server will return a ranked list of significant matches, along with an alignment of the query sequence to the matched BLOCKS entries.

[0270] Conserved protein domains can be represented by two-dimensional matrices, which measure either the frequency or probability of the occurrences of each amino acid residue and deletions or insertions in each position of the domain. This type of model, when used to search against protein databases, is sensitive and usually yields more accurate results than simple motif searches. Two popular implementations of this approach are profile searches (such